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Proteomic analysis of nitrate-dependent acetone degradation by *Alicyclophilus denitrificans* strain BC

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One sentence summary: We provide insight in bacterial nitrate-dependent degradation of acetone, which contributes to understanding anaerobic microbial removal of hazardous acetone from the environment.

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ABSTRACT

Alicyclophilus denitrificans strain BC grows anaerobically on acetone with nitrate as electron acceptor. Comparative proteomics of cultures of *A. denitrificans* strain BC grown on either acetone or acetate with nitrate was performed to study the enzymes involved in the acetone degradation pathway. In the proposed acetone degradation pathway, an acetone carboxylase converts acetone to acetoacetate, an AMP-dependent synthetase/ligase converts acetoacetate to acetoacetyl-CoA, and an acetyl-CoA acetyltransferase cleaves acetoacetyl-CoA to two acetyl-CoA. We also found a putative aldehyde dehydrogenase associated with acetone degradation. This enzyme functioned as a β -hydroxybutyrate dehydrogenase catalyzing the conversion of surplus acetoacetate to β -hydroxybutyrate that may be converted to the energy and carbon storage compound, poly- β -hydroxybutyrate. Accordingly, we confirmed the formation of poly- β -hydroxybutyrate in acetone-grown cells of strain BC. Our findings provide insight in nitrate-dependent acetone degradation that is activated by carboxylation of acetone. This will aid studies of similar pathways found in other microorganisms degrading acetone with nitrate or sulfate as electron acceptor.

Keywords: *Alicyclophilus denitrificans*; acetone; proteome; anaerobic; nitrate; β -hydroxybutyrate

INTRODUCTION

Acetone (dimethylketone, 2-propanone) is a compound that is formed by the cumene process (Hock and Lang 1944), but also by anaerobic fermentation using microorganisms such as *Clostridium acetobutylicum* (Davies and Stephenson 1941; George et al. 1983). Acetone is volatile and present in the upper troposphere where it may contribute to the production of hydrogen-oxygen

radicals (i.e. HO and HO₂) and to nitrogen oxide and ozone cycling (Singh et al. 1995). It is also a common organic solvent widely used in production of industrial chemicals such as cosmetics, drugs, vitamins and as a disinfectant (Inchem 1998). Large-scale disposal of hazardous acetone by pharmaceutical, electronic and chemical industries has put a burden to the environment, necessitating its microbial removal

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as a safe approach (Balasubramanian, Philip and Bhallamudi 2011).

Acetone can be degraded by aerobic as well as anaerobic microorganisms. Several pathways for aerobic microbial acetone degradation are known. Four isolated *Corynebacterium* strains have been described that can aerobically convert acetone to 1-hydroxyacetone by acetone monooxygenase which is further metabolized to methylglyoxal and pyruvate (Taylor et al. 1980). Pyruvate is further metabolized to acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle. Another aerobic acetone degradation pathway was shown to be active in *Gordonia* sp. strain TY-5. Here, acetone is oxidized to methyl acetate by acetone monooxygenase and a subsequent monooxygenase mediated conversion of methyl acetate to acetate and methanol (Kotani et al. 2007). Acetate is further degraded in the TCA cycle; however, the fate of methanol is unknown.

Anaerobically acetone can be carboxylated to acetoacetate (acetone + CO₂ + ATP + 2H₂O → acetoacetate + AMP + 2P_i + 3H⁺). Acetoacetate is activated to acetoacetyl-CoA and then converted to two acetyl-CoA (Platen, Temmes and Schink 1990). The initial activation of acetone is known to be catalyzed by an acetone carboxylase, but the other enzymes are unknown. Acetone carboxylase is involved in anaerobic photosynthetic degradation of acetone, and in acetone degradation with nitrate or sulfate as electron acceptor (Siegel 1957; Taylor et al. 1980; Bonnet-Smits et al. 1988; Platen and Schink 1989; Birks and Kelly 1997; Sluis et al. 2002; Dullius, Chen and Schink 2011). Recently, a novel mechanism for acetone degradation in sulfate-reducing bacteria was proposed (Gutiérrez Acosta, Hardt and Schink 2013). Here, acetone is carbonylated to acetoacetaldehyde, which is subsequently converted to acetoacetyl-CoA and to acetyl-CoA. Acetone carbonylation seems to be ATP- and thiamine diphosphate-dependent (Gutiérrez Acosta, Schleheck and Schink 2014).

The carboxylation of acetone is an ATP-dependent and thermodynamically unfavorable reaction ($\Delta G^0 = +17.1 \text{ kJ mol}^{-1}$) (Ensign et al. 1998; Schühle and Heider 2012). In the unidentified denitrifying bacterium BunN, ADP-dependent decarboxylation of acetoacetate is catalyzed by acetone carboxylase (Janssen and Schink 1995). Previously the acetone carboxylase of *Alicyclophilus denitrificans* strain KN Bun08 and *A. denitrificans* strain K601^T were purified and characterized (Dullius, Chen and Schink 2011). Here, we studied acetone degradation by *A. denitrificans* strain BC using nitrate as the electron acceptor and performed proteome analysis to obtain insight into its acetone degradation pathway.

MATERIALS AND METHODS

Physiological studies and culture preparation

Alicyclophilus denitrificans strain BC (DSM 18852) was isolated in our laboratory and grown in an anaerobic phosphate-bicarbonate-buffered (pH 7.3) AW1-sulfate medium (Weelink et al. 2007, 2008). Cultures were incubated without agitation at 30°C in the dark.

Growth of strain BC with acetone as electron donor and either nitrate, chlorate or oxygen as the electron acceptor was tested using 40 mL medium in 120-mL serum flasks with N₂ and CO₂ (80:20%, 170 kPa) as the gas phase. Different concentrations of acetone (2, 4 or 8 mM) were used with acetate added in low concentration (1 mM) to initiate growth. Nitrate and chlorate were added to final concentrations of 10 and 3 mM (4 mL per flask) of oxygen was added to the headspace from a sterile pure oxygen stock. A 5% (v/v) of inoculum was added to these cultures. Sim-

ilar cultures with 10 mM acetate were used as positive controls and uninoculated bottles were used as negative control. Cultures were generally incubated for six days.

For physiological analysis, strain BC was grown in triplicate with acetone (10 mM) or acetate (10 mM) and nitrate (10 mM). A 5% (v/v) of cells was used as inoculum. Cultures were grown in 120-mL bottles as described above and sampled at 0, 2, 5 and 8 days of incubation. For growth analysis, 1.5-mL culture samples were used for OD₆₆₀ measurements with a spectrophotometer (Hitachi, Tokyo, Japan). Afterwards, the samples were stored at -20°C, prior to liquid chromatography analysis. To determine biomass yields, 30 mL of late-log cultures was harvested in 50-mL Greiner tubes (Greiner Bio-One, Frickenhausen, Germany) and centrifuged at 4700 RPM for 3 min in Eppendorf centrifuge 5810R (Eppendorf, Hamburg, Germany). Pellets were dried in a SpeedVac concentrator (Savant Instruments, Holbrook, NY, USA) at room temperature for 15 min, and the weight of the pellets was determined. The dry weight in g L⁻¹ was calculated from the weight per 30 mL. The amount of electron donor (in mmol L⁻¹) that was used by the cells was determined using liquid chromatography, and this was used to calculate the dry weight biomass in g (mol electron donor)⁻¹.

To obtain cells for proteome analysis, 80-mL cultures were grown in 250-mL flasks with acetone or acetate (10 mM), nitrate (10 mM) and 5% inoculum (cells used as inoculum were adapted to acetone and nitrate or acetate and nitrate).

Gas chromatography

Oxygen was measured by headspace analysis using a gas chromatograph (GC-14B; Shimadzu, Kyoto, Japan) with a packed column (Molsieve 13 × 60/80 mesh, 2 m × 2.4 mm; Varian, Mid-delburg, the Netherlands) equipped with a thermal conductivity detector. The column, detector and injector temperatures were 100, 150 and 90°C, respectively. Argon was used as carrier gas (the flow rate was 30 mL min⁻¹). At each analysis interval, 0.4 mL headspace was injected.

Liquid chromatography

The anions chlorate, nitrate, chloride and nitrite were measured by suppressor mediated ion chromatography (Dionex, Breda, the Netherlands) equipped with an IonPac AS9-SC column (Dionex) and a conductivity detector. An eluent containing 1.8 mM Na₂CO₃ and 1.7 mM NaHCO₃ was pumped at a flow rate of 1 mL min⁻¹. Mannitol (10 mM) was added to samples for stabilization, and potassium fluoride (1 mM) was used as internal standard. The liquid chromatograph used for acetone and acetate analysis was equipped with a column for detection of organic acids (Merck organic acid column 300-6.5: Polyspher OA HY). The eluent was 10 mN sulfuric acid and used at a flow rate of 0.8 mL min⁻¹. The internal standard was 10 mM crotonate.

Poly-β-hydroxybutyrate (PHB) and Gram staining

Cells of *A. denitrificans* were grown with acetate and nitrate (both 10 mM) and transferred to acetone or acetate and nitrate (all 10 mM). PHB and Gram staining were performed as previously described (Wei et al. 2011). Briefly, smears of cultures were prepared on glass slides and heat fixed. The slides were stained with a 0.3% Sudan Black (w/v in 70% ethanol, Sigma) solution for 10 min followed by decolorization in xylene. The slides were then counterstained with 0.5% Safranin for 10 s,

washed with distilled water, dried and examined by optical microscopy (Leica DM2000).

Proteomics

Cell-free extracts of strain BC grown to end-log phase with either acetate or acetone and nitrate were prepared for whole-proteome analysis and as described previously (Wolterink et al. 2002). Protein concentrations were determined using a Bio-rad protein assay (Bio-rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Bovine serum albumin was used as protein standard. Then 25 µg of protein was loaded on a 10% SDS-polyacrylamide separation gel using the mini-protean 3 cell. Electrophoresis and gel staining using Coomassie Brilliant Blue R-250 were performed according to the mini-protean 3 cell manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA, USA). Gels were scanned, and similarity of the intensity of each lane was checked using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

SDS-PAGE separated proteins from strain BC grown with either acetate or acetone and nitrate were subjected to in-gel digestion. Gel lanes were divided into four slices using a scalpel and processed to pieces of about 1 mm². Gel pieces were reduced, alkylated and trypsin digested as previously described (Rupakula et al. 2013). The supernatant was used for LC-MS/MS analysis.

Protein digests obtained from cells of *A. denitrificans* strain BC grown with either acetate or acetone and nitrate were analyzed on LC-MS/MS as described previously (Lu et al. 2011). MS/MS spectra were analyzed using MaxQuant software (<http://maxquant.org>) and a protein database of *A. denitrificans* strain BC obtained from the European Bioinformatics Institute (www.ensemblgenomes.org), as described before with a false discovery rate of maximally 1% on protein and peptide level and filtering out proteins identified by less than two peptides as well as those with no unique and no unmodified peptide (Peng, Van Lent and Boeren 2012). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository with the dataset identifier PXD000258 (Vizcaíno et al. 2013).

Phylogeny of the alpha subunit of acetone carboxylases

The predicted amino acid sequence of the acetone carboxylase alpha subunit of strain BC (Alide.1503) was used for BLASTp analysis (Altschul et al. 1997; Oosterkamp et al. 2011). The most related proteins (11 taxons) were selected and used for clustalW alignment and phylogenetic tree construction (www.ebi.ac.uk/Tools/msa/clustalw2 and [Tools/phylogeny](http://www.ebi.ac.uk/Tools/phylogeny)). The phylogenetic tree was calculated using the neighbor-joining clustering method in nexus tree format. Using these data, a tree was constructed in TreeView (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>).

RESULTS AND DISCUSSION

Nitrate-dependent acetone degradation by *A. denitrificans* strain BC

Growth of *A. denitrificans* strain BC with acetone as electron donor and nitrate, chlorate or oxygen as electron acceptor was compared to control experiments with acetate as electron donor. While all electron acceptors supported growth on ac-

etate, growth on acetone was only observed with nitrate (data not shown). Oxygenases have been described to mediate aerobic degradation of acetone (Taylor et al. 1980; Kotani et al. 2007). The genome of strain BC contains several oxygenases that can function in presence of oxygen and chlorate (Oosterkamp et al. 2011). However, here they do not seem to be involved in acetone conversion as no growth was observed with either oxygen or chlorate (data not shown).

Although acetate was consumed faster than acetone with nitrate, the optical density in acetone-grown cultures was about twice as high (Fig. 1). Furthermore, the dry weight biomass yield per mol of electron donor used was about two and a half times higher for acetone-grown cells compared to acetate-grown cells (Table 1). The biomass yields of *A. denitrificans* strain KN Bun08, *Paracoccus pantotrophus* and *Paracoccus denitrificans*, were about 20, 28 and 28 g mol⁻¹ acetone and 9, 14 and 12 g mol⁻¹ acetate, respectively (Dullius, Chen and Schink 2011). Furthermore, the growth yield of the unidentified strain BunN was about 27 g mol⁻¹ acetone (Platen and Schink 1989). With the yield of 39 g mol⁻¹ acetone, strain BC (Table 1) has the highest biomass yield per mol acetone. Moreover, the Gibbs free energy change of acetone oxidation coupled to nitrate reduction is about two times higher than of acetate oxidation coupled to nitrate reduction ($C_3H_6O + 3.2 NO_3^- + 3.2 H^+ \rightarrow 3 CO_2 + 1.6 N_2 + 8.6 H_2O$ and $-1614 kJ mol^{-1}$ versus $C_2H_3O_2 + 2.6 H^+ + 1.6 NO_3^- \rightarrow 2 CO_2 + 0.8 N_2 + 2.8 H_2O$ and $-792 kJ mol^{-1}$). Accordingly, the theoretical electron donor to electron acceptor ratio is 1:3.2 for acetone and nitrate and 1:2.6 for acetate and nitrate and our experimental data gave 1:1.9 and 1:1.3 ratios for acetone:nitrate and acetate:nitrate, respectively. The experimental ratios are lower than the theoretical ratios, which partly can be explained by conversion of electron donors to biomass.

Proteomics of *A. denitrificans* strain BC grown with acetone and acetate

Proteomes from *A. denitrificans* strain BC grown with acetone or acetate and nitrate as electron acceptor were compared to identify enzymes involved in acetone degradation. As can be seen in Fig. 2, the three acetone carboxylase subunits acxABC (encoded by Alide.1502 to 1504) were abundant in cells grown with acetone. This enzyme is about 4000-fold more abundant in acetone-grown cells than in acetate-grown cells (Table 2). Acetone carboxylase of *A. denitrificans* strain KN Bun08 is a hexamer with an $\alpha_2\beta_2\gamma_2$ -structure (Dullius, Chen and Schink 2011). In the genome of strain BC, Alide.1504 was annotated as acetone carboxylase gamma subunit (Oosterkamp et al. 2011). The amino acid sequences of direct adjacent proteins encoded by Alide.1502 and Alide.1503 are highly similar to the sequences of beta and alpha acetone carboxylase subunits from *A. denitrificans* strain KN Bun08 (Dullius, Chen and Schink 2011). In the genome of strain BC, these genes were previously misannotated as enzymes from the same protein family as acetone carboxylases (Pfam01968), hydantoinase/oxoprolinase and hydantoinase b/oxoprolinase, respectively (Oosterkamp et al. 2011; Finn et al. 2014).

Acetone carboxylase converts acetone to acetoacetate, which is further degraded to acetoacetyl-CoA and then to two acetyl-CoA (Platen, Temmes and Schink 1990). In acetone-grown cells of strain BC, an AMP-dependent synthetase/ligase (Alide.4154) was 65-fold more abundant than in acetate-grown cells (Fig. 2, Table 2). CoA transferases, such as acetoacetate-CoA ligase and acetyl-CoA synthetase require binding of AMP to induce a conformational change in the active site that allows the

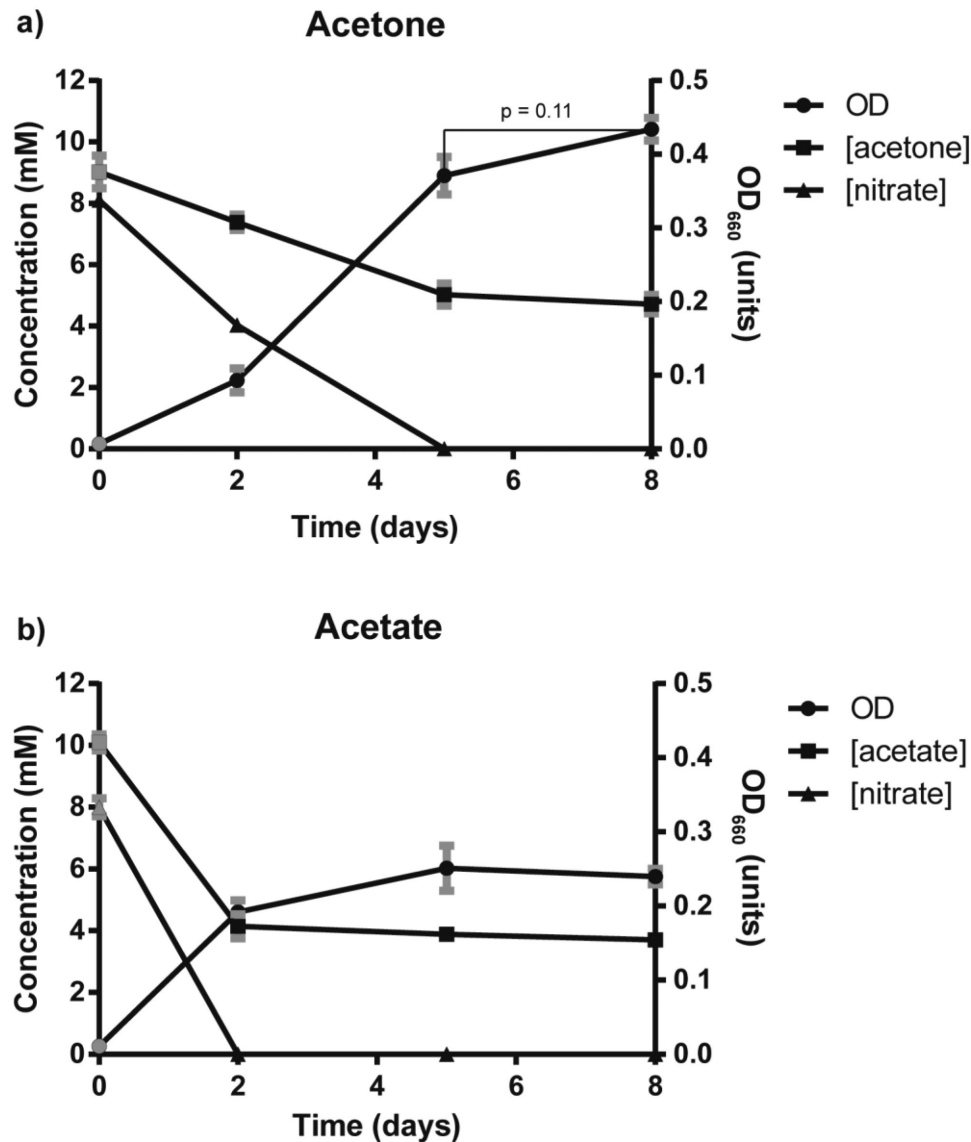


Figure 1. Growth of *A. denitrificans* strain BC on acetone (a) and acetate (b) with nitrate as electron acceptor shown as the optical density (OD at 660 nm) and the concentrations of acetone or acetate and nitrate. A paired t-test was used to determine the P value (significant at $0.05 < p < 0.95$).

Table 1. Characteristics of *A. denitrificans* strain BC grown with acetone and acetate as electron donor and nitrate as electron acceptor shown as the changes in culture density, electron donor concentration, electron acceptor concentration and as the dry weight yield.

Culture	Δ culture density (OD units)	Δe^- donor (mM)	Δe^- acceptor (mM)	Dry weight yield (gram per mol e^- donor)
Acetone	0.427 ± 0.016	4.30 ± 0.23	8.11 ± 0.15	39.0 ± 34.3
Acetate	0.229 ± 0.016	6.39 ± 0.16	8.00 ± 0.29	12.9 ± 2.3

binding of CoA to the substrate (Jogl and Tong 2004). Likely, the AMP-dependent synthetase/ligase (Alide_4154) functions as acetoacetate-CoA ligase. An acetyl-CoA acetyltransferase (Alide_0678) that can convert acetoacetyl-CoA to two acetyl-CoA was 68-fold more abundant in acetone-grown cells (Fig. 2, Table 2).

Interestingly, an aldehyde dehydrogenase (Alide_4113) was highly abundant in acetone-grown cells (Fig. 2, Table 2). We speculate that this enzyme is a β -hydroxybutyrate dehydro-

genase that catalyzes the conversion of acetoacetate to β -hydroxybutyrate. PHB formation during growth with acetone has been previously described under some growth conditions (for example nitrogen limitation) in other anaerobic bacteria such as *Thiosphaera pantotropha* and *Desulfococcus biacutus* (Bonnet-Smits et al. 1988; Platen, Temmes and Schink 1990). Our hypothesis for strain BC was confirmed by staining for PHB in acetone-grown cells and acetate-grown cells (Fig. S1, Supporting Information). Proteomic analysis of early- and end-log-phase

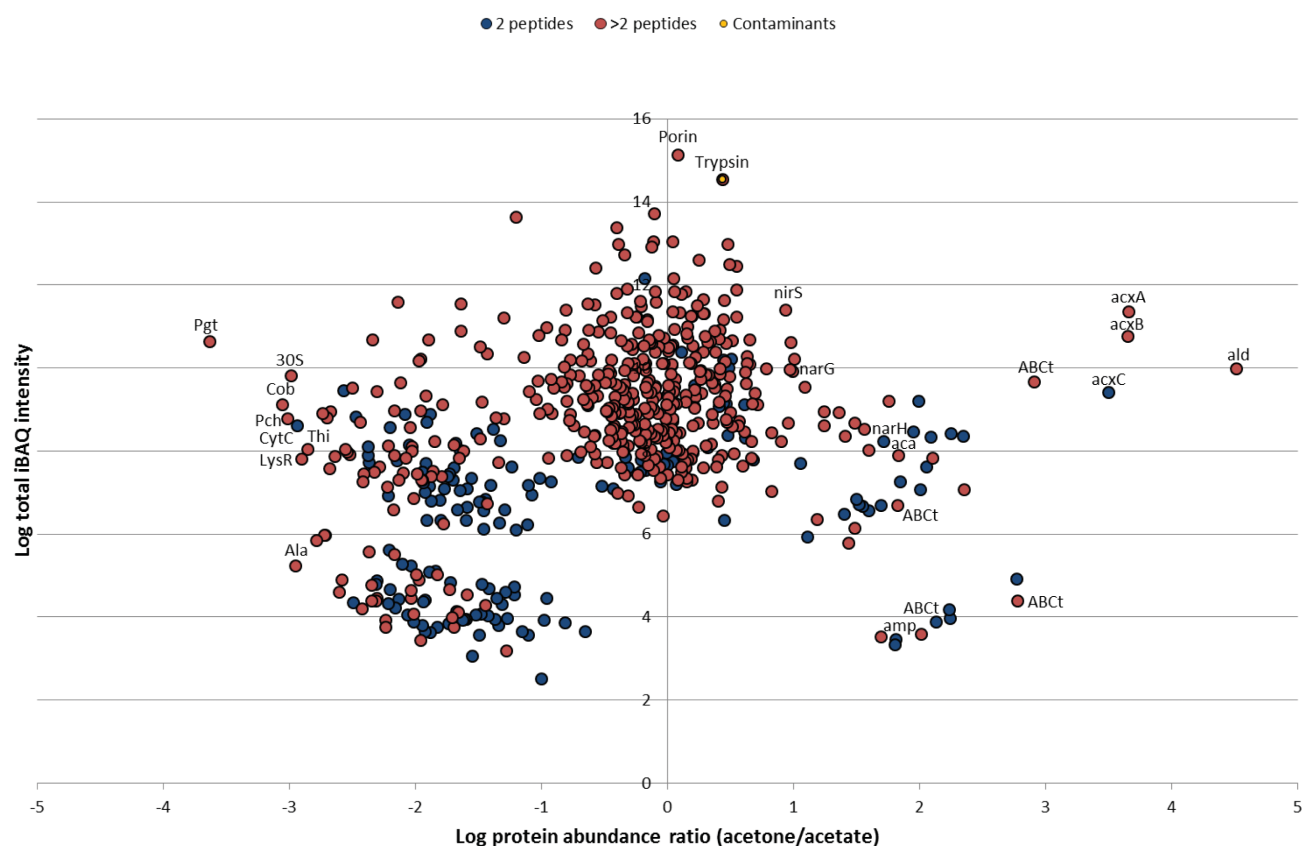


Figure 2. Protein abundance in *A. denitrificans* strain BC grown with acetone and nitrate or with acetate and nitrate. Proteins were detected by LC-MS/MS and quantified using MaxQuant software. The log total iBAQ intensity is plotted against the log protein abundance ratio of acetone to acetate. Acetone carboxylase (acxABC), aldehyde dehydrogenase (ald), ABC transporter (ABCT), acetyl-CoA acetyltransferase (aca), AMP-dependent synthetase/ligase (amp), nitrate reductase (narG), nitrite reductase (nirS) and trypsin are indicated.

cells grown with acetone (without and with nitrogen limitation) and the determination of the specific activity and coenzyme preference of the proposed β -hydroxybutyrate dehydrogenase may be worthwhile to further study PHB formation in strain BC.

Other proteins that were abundantly present in acetone-grown cells are an extracellular ligand-binding ABC transporter (Alide_1344–1347), regulators (Alide_0251+0468+2994+3200+3351) and transporters (Alide_0395+0650+2304) (Table 2). Proteins that were more abundant in acetate-grown cells include peptidoglycan glycosyltransferase, cobaltochelatase, protein channel protein and ribosomal proteins (Fig. 2, Table 2).

Phylogenetic analysis of *A. denitrificans* strain BC acetone carboxylase

An important enzyme in the acetone metabolism of *A. denitrificans* strain BC is acetone carboxylase. We compared gene and protein sequences of the acetone carboxylase of strain BC to those of strain K601^T (Dullius, Chen and Schink 2011). Homologs of the genes coding for the acetone carboxylase alpha, beta and gamma subunit of strain BC (Alide_1503, 1502 and 1504) are also present in strain K601^T (Alide_2.3423, 3424 and 3422). The alpha subunit encoding genes differ at a nucleotide position 1657 (G (strain BC) / A (strain K601^T)) that leads to a non-synonymous mutation (T553A). Genes encoding the acetone carboxylase beta subunit are identical and, there is a single synonymous mu-

tation in the carboxylase gamma subunit encoding genes (nucleotide 66 is T in strain BC and C in strain K601^T).

The catalytic subunit of the acetone carboxylase (alpha subunit, Alide_1503) of strain BC was subsequently used for a phylogenetic comparison with closely related proteins from Betaproteobacteria, including *Azoarcus*, *Thauera*, *Ralstonia*, *Dechloromonas*, *Cupriavidus* and *Methyloversatilis* species (Fig. S2, Supporting Information). Of these bacteria, *Methyloversatilis universalis* strain FAM5^T (Kalyuzhnyaya et al. 2006) and *Cupriavidus metallidurans* strain CH34 (Makkar and Casida 1987; Rosier et al. 2012) are known to degrade acetone. Moreover, some were described to contain acetone carboxylase genes, i.e. *Thauera* sp MZ1T (Lajoie et al. 2000; Rosier et al. 2012), *Ralstonia eutropha* strain JMP134 (also known as *Cupriavidus pinatubonensis* strain JMP134) (Rosier et al. 2012), *Dechloromonas aromatica* strain RCB and *Azoarcus aromaticum* strain EbN1 (Rosier et al. 2012). The other bacteria that contained a protein closely related to the alpha subunit of acetone carboxylase of strain BC, *Azoarcus communis* (Reinhold-Hurek et al. 1993), *Cupriavidus necator* strain N-1 and *Azoarcus* sp. strain KH32C (Tago et al. 2011), are not known to degrade acetone. All of these Betaproteobacteria may have a similar acetone degradation pathway.

In conclusion, comparative proteomics of cultures of *A. denitrificans* strain BC grown on acetone with nitrate revealed an anaerobic pathway involved in the acetone degradation pathway. In the proposed acetone degradation pathway, an acetone carboxylase converts acetone to acetoacetate, an AMP-dependent synthetase/ligase converts acetoacetate to

Table 2. Most abundant proteins from the comparison of the proteomes of *A. denitrificans* strain BC grown on acetone or acetate with nitrate. Cut-off was set to log protein abundances <-2.8 and >1.8 . The log total iBAQ intensity and log protein abundance ratio are as obtained from MaxQuant analysis. Uniprot numbers and protein descriptions are obtained from the EBI protein database.

Protein description	Gene #	Uniprot #	Log protein abundance ratio	Log total iBAQ intensity	Δ abundance acetone to acetate (fold)
Aldehyde dehydrogenase	Alide_4113	E8TW43	4.516	9.989	32810
Acetone carboxylase A	Alide_1503	E8TTA6	3.663	11.357	4603
Acetone carboxylase B	Alide_1502	E8TTA5	3.652	10.764	4487
Acetone carboxylase C	Alide_1504	E8TTA7	3.501	9.419	3170
ABC transporter extracellular ligand	Alide_1346	E8TRK6;E8U068	2.910	9.669	813
ABC transporter extracellular ligand	Alide_1347	E8TRK7	2.782	4.395	605
Helix-turn-helix domain regulator	Alide_0251	E8TTT1	2.770	4.923	589
Chemotaxis sensory transducer	Alide_2994	E8TWT0	2.353	7.075	225
Efflux transporter	Alide_2304	E8U1Q1	2.346	8.361	222
LemA family protein	Alide_0447	E8TVL0	2.252	8.424	179
Protein of unknown function	Alide_0553	E8TX56	2.245	3.972	176
Response regulator receiver	Alide_0468	E8TWA3	2.238	4.184	173
ABC transporter extracellular ligand	Alide_1344	E8TRK4	2.132	3.886	136
Chemotaxis sensory transducer	Alide_3351	E8U0M0	2.108	7.823	128
Cytb containing protein	Alide_3380	E8U0P9	2.088	8.340	122
Basic membrane lipoprotein	Alide_1365	E8TRM5	2.054	7.611	113
Dead/deah box helicase domain protein	Alide_1216	E8TQ43	2.014	3.579	103
Heat-shock chaperone protein	Alide_0582	E8TX83	2.008	7.073	102
Ribosomal interface protein	Alide_4171	E8TW99	1.994	9.205	99
Sporulation domain-containing protein	Alide_4085	E8TVE4	1.954	8.466	90
ABC transporter extracellular ligand	Alide_1345	E8TRK5	1.846	7.261	70
Acetyl-CoA acetyltransferase	Alide_0678	E8TY47	1.831	7.892	68
Flagellin domain protein	Alide_3863	E8TSX0	1.830	6.696	68
AMP-dependent synthetase/ligase	Alide_4154	E8TW82	1.815	3.458	65
PAS sensor protein	Alide_3200	E8TZA1	1.804	3.326	64
Thiazole biosynthesis family protein	Alide_1783	E8TWL1	-2.855	8.031	-716
LysR family transcriptional regulator	Alide_2752	E8TU78	-2.905	8.602	-804
Cytochrome-c oxidase	Alide_3609	E8TQH0	-2.938	5.231	-867
Alanine racemase	Alide_3409	E8U0S8	-2.953	9.823	-897
Ribosomal protein s6	Alide_3124	E8TYE8	-2.988	8.788	-973
Proton channel subunit	Alide_2521	E8TRP0	-3.014	9.118	-1033
Cobaltochelatase	Alide_1228	E8TQL5	-3.054	10.632	-1132
Peptidoglycan glycosyltransferase	Alide_2254	E8U152	-3.635	5.231	-4315

acetoacetyl-CoA and an acetyl-CoA acetyltransferase cleaves acetoacetyl-CoA to two acetyl-CoA. We suggest that surplus acetoacetate is converted to β -hydroxybutyrate by a putative aldehyde dehydrogenase that may be converted to the energy and carbon storage compound, PHB. Accordingly, we confirmed the formation of PHB in acetone-grown cells of strain BC.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSLE online.

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Conflict of interest. None declared.

REFERENCES

- Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.
- Balasubramanian P, Philip L, Bhallamudi SM. Biodegradation of chlorinated and non-chlorinated VOCs from pharmaceutical industries. *Appl Bioc Biotechnol* 2011;**163**:497–518.
- Birks SJ, Kelly DJ. Assay and properties of acetone carboxylase, a novel enzyme involved in acetone-dependent growth and CO₂ fixation in *Rhodobacter capsulatus* and other photosynthetic and denitrifying bacteria. *Microbiology* 1997;**143**:755–66.
- Bonnet-Smits EM, Robertson LA, Van Dijken JP, et al. Carbon dioxide fixation as the initial step in the metabolism of acetone by *Thiosphaera pantotropha*. *J Gen Microbiol* 1988;**134**:2281–9.
- Davies R, Stephenson M. Studies on the acetone-butyl alcohol fermentation. *Biochem J* 1941;**35**:1320–31.
- Dullius CH, Chen CY, Schink B. Nitrate-dependent degradation of acetone by *Alicyclophilus* and *Paracoccus* strains and comparison of acetone carboxylase enzymes. *Appl Environ Microb* 2011;**77**:6821–5.

- Ensign SA, Small FJ, Allen JR, et al. New roles for CO₂ in the microbial metabolism of aliphatic epoxides and ketones. *Arch Microbiol* 1998;169:179–87.
- Finn RD, Bateman A, Clements J, et al. Pfam: the protein families database. *Nucleic Acids Res* 2014;42:D222–30.
- George HA, Johnson JL, Moore WE, et al. Acetone, isopropanol, and butanol production by *Clostridium beijerinckii* (syn. *Clostridium butylicum*) and *Clostridium aurantibutyricum*. *Appl Environ Microb* 1983;45:1160–3.
- Gutiérrez Acosta OB, Hardt N, Schink B. Carbonylation as a key reaction in anaerobic acetone activation by *Desulfococcus biacutus*. *Appl Environ Microb* 2013;79:6228–35.
- Gutiérrez Acosta OB, Schleheck D, Schink B. Acetone utilization by sulfate-reducing bacteria: draft genome sequence of *Desulfococcus biacutus* and a proteomic survey of acetone-inducible proteins. *BMC Genomics* 2014;15:584.
- Hock H, Lang S. Autoxydation von kohlenwasserstoffen, IX. mitteil.: über peroxyde von benzol-derivaten. *Ber Dtsch Chem Ges* 1944;77:257–64.
- Inchem (International Programme on Chemical Safety). *Environmental Health Criteria*, 207: Acetone. Geneva:World Health Organization, 1998, <http://www.inchem.org>.
- Janssen PH, Schink B. 14CO₂ exchange with acetoacetate catalyzed by dialyzed cell-free extracts of the bacterial strain BunN grown with acetone and nitrate. *Eur J Biochem* 1995;228:677–82.
- Jogl G, Tong L. Crystal structure of yeast acetyl-Coenzyme A synthetase in complex with AMP. *Biochemistry* 2004;43:1425–31.
- Kalyuzhnaya MG, De Marco P, Bowerman S, et al. *Methyloversatilis universalis* gen. nov., sp. nov., a novel taxon within the Betaproteobacteria represented by three methylotrophic isolates. *Int J Syst Evol Micr* 2006;56:2517–22.
- Kotani T, Yurimoto H, Kato N, et al. Novel acetone metabolism in a propane-utilizing bacterium, *Gordonia* sp. strain TY-5. *J Bacteriol* 2007;189:886–93.
- Lajoie CA, Layton AC, Gregory IR, et al. Zoogeal clusters and sludge dewatering potential in an industrial activated-sludge wastewater treatment plant. *Water Environ Res* 2000;72:56–64.
- Lu J, Boeren S, De Vries SC, et al. Filter-aided sample preparation with dimethyl labeling to identify and quantify milk fat globule membrane proteins. *J Proteomics* 2011;75:34–43.
- Makkar NS, Casida LE. *Cupriavidus necator* gen. nov., sp. nov.; a nonobligate bacterial predator of bacteria in soil. *Int J Syst Bacteriol* 1987;37:323–6.
- Oosterkamp MJ, Veuskens T, Plugge CM, et al. Genome sequences of *Alicyciphilus denitrificans* strains BC and K601^T. *J Bacteriol* 2011;193:5028–9.
- Peng K, Van Lent JWM, Boeren S, et al. Characterization of novel components of the baculovirus per os infectivity factor complex. *J Virol* 2012;86:4981–8.
- Platen H, Schink B. Anaerobic degradation of acetone and higher ketones via carboxylation by newly isolated denitrifying bacteria. *J Gen Microbiol* 1989;135:883–91.
- Platen H, Temmes A, Schink B. Anaerobic degradation of acetone by *Desulfococcus biacutus* spec. nov. *Arch Microbiol* 1990;154:355–61.
- Reinhold-Hurek B, Hurek T, Gillis M, et al. *Azoarcus* gen. nov., nitrogen-fixing Proteobacteria associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth), and description of two species, *Azoarcus indigenus* sp. nov. and *Azoarcus communis* sp. nov. *Int J Syst Bacteriol* 1993;43:574–84.
- Rosier C, Leys N, Henoumont C, et al. Purification and characterization of the acetone carboxylase of *Cupriavidus metallidurans* strain CH34. *Appl Environ Microb* 2012;78:4516–8.
- Rupakula A, Kruse T, Boeren S, et al. The restricted metabolism of the obligate organohalide respiring bacterium *Dehalobacter restrictus*: lessons from tiered functional genomics. *Philos T R Soc B* 2013;368:20120325.
- Schühle K, Heider J. Acetone and butanone metabolism of the denitrifying bacterium ‘*Aromatoleum aromaticum*’ demonstrates novel biochemical properties of an ATP-dependent aliphatic ketone carboxylase. *J Bacteriol* 2012;194:131–41.
- Siegel JM. The dark anaerobic metabolism of acetone and acetate by the photosynthetic bacterium *Rhodospseudomonas gelatinosa*. *J Biol Chem* 1957;228:41–7.
- Singh HB, Kanakidou M, Crutzen PJ, et al. High concentrations and photochemical fate of oxygenated hydrocarbons in the global troposphere. *Nature* 1995;378:50–4.
- Sluis MK, Larsen RA, Krum JG, et al. Biochemical, molecular, and genetic analyses of the acetone carboxylases from *Xanthobacter autotrophicus* strain Py2 and *Rhodobacter capsulatus* strain B10. *J Bacteriol* 2002;184:2969–77.
- Tago K, Ishii S, Nishizawa T, et al. Phylogenetic and functional diversity of denitrifying bacteria isolated from various rice paddy and rice-soybean rotation fields. *Microb Environ* 2011;26:30–5.
- Taylor DG, Trudgill PW, Cripps RE, et al. The microbial metabolism of acetone. *J Gen Microbiol* 1980;118:159–70.
- Vizcaino JA, Côté RG, Csordas A, et al. The PRoteomics IDentifications (PRIDE) database and associated tools: status in 2013. *Nucleic Acids Res* 2013;41:D1063–9.
- Weelink SA, Tan NC, Ten Broeke H, et al. Physiological and phylogenetic characterization of a stable benzene-degrading, chlorate-reducing microbial community. *FEMS Microbiol Ecol* 2007;60:312–21.
- Weelink SA, Tan NC, Ten Broeke H, et al. Isolation and characterization of *Alicyciphilus denitrificans* strain BC that grows on benzene with chlorate as electron acceptor. *Appl Environ Microb* 2008;72:6672–81.
- Wei YH, Chen WC, Huang CK, et al. Screening and evaluation of polyhydroxybutyrate-producing strains from indigenous isolate *Cupriavidus taiwanensis* strains. *Int J Mol Sci* 2011;12:252–65.
- Wolterink AF, Jonker AB, Kengen SW, et al. *Pseudomonas chloritidismutans* sp. nov., a non-denitrifying, chlorate-reducing bacterium. *Int J Syst Evol Micr* 2002;52:2183–90.